



The Biological, Biochemical, and Immunological Impact of Broccoli and Green Pea Sprouts on Acrylamide Intoxicated Rats

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Abstract

Consuming plant sprouts as part of the daily human diet is slowly on the increase, and the health benefits are now of interest in several disciplines. The aim of this investigation was to evaluate the chemical compositions of dried broccoli or dried green pea sprouts and the subsequent effects on the biological, biochemical and immunological parameters of male albino rats supplemented with acrylamide as a toxic material. Chemical constituents, mineral contents, total phenols and total flavonoid of tested vegetable sprouts were analyzed. Thirty male albino rats weighting 160 ± 5 g. were separated into 6 groups. The negative control group was first, and then the second group was given acrylamide $2.5 \mu\text{g}/\text{kg}/\text{day}$. Groups (3, 4) and (5,6) were given treatment of the same toxic material and received 5 and 10 g dried broccoli and pea sprouts per 100 g basal diet, respectively. Feed intake, body weight gain, FER and some organs weights were calculated towards the conclusion of the investigation. Also, liver enzymes, lipids profile, serum glucose, kidney function, brain enzymes, oxidative activity and immunoglobulin were determined. Results showed that tested sprouts had high protein, fiber, vital minerals, total phenols and total flavonoid components. In addition, there were significant positive effects on the biochemical parameters that were tested, such as lipids profile, serum glucose, liver function, kidney functions, oxidative activity, brain enzyme and immunity responses, particularly at the 10% level, of tested sprouts and pea sprouts had high effect on tested parameters more than broccoli sprouts. In conclusion, pea and broccoli



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sprouts could potentially be used as protective agents against the toxic effect of acrylamide and the obtained results suggested that tested sprouts can be used as ideal candidates of natural antioxidant agents.

Introduction

As a commodity chemical, acrylamide has numerous laboratory and industrial applications. After being cooked at high temperatures, many starchy foods naturally produce acrylamide through chemical reactions. Potato chips, French fries, grains (including breakfast cereals, toast, and cookies), coffee, and foods produced from grains are a few examples of foods with higher levels of acrylamide.¹ When reducing, sugars (fructose or glucose) and the amino acid asparagine interact as part of the Maillard reaction, which is the mechanism that causes food to brown during roasting, frying, and baking; acrylamide is produced as a cooking process byproduct. Thus, elements such as cooking duration and temperature, moisture content, and the quantity of reducing asparagine and sugar in raw foods all have an impact on acrylamide levels in cooked food.² Health risks to the general population based on an average exposure of $1 \mu\text{g kg}^{-1}$ body weight per day, increased to $4 \mu\text{g kg}^{-1}$ body weight per day for high consumers. Several epidemiological studies have performed an evaluation of the potential association with neurotoxicity as having the main toxic impact after occupational exposure. It is also toxic for reproduction, affecting male germ cells and being carcinogenic to a number of organs, including reproductive organs, gastrointestinal tract, kidneys, lungs, and brain.^{3,4}

Of any food evaluated, a sprout has an almost complete nutritional profile. Sprouts are full of vitamins, minerals, enzymes, and proteins. The ancient Egyptians and Chinese were aware of the nutritional benefits thousands of years ago, whereas seed germination and sprout production were used by the ancients. Sprouts are an organic food that improve seed quality through enhancing digestibility and decreasing the concentration of resistant starch and anti-nutritive chemicals.⁵

Broccoli sprouts (*Brassica oleracea L. var. italica*) have small green leaves and short white stems that are connected to the seed. Broccoli sprouts

are nutritious powerhouses, high in protein, fiber, vitamins (A and C), minerals (calcium and iron), and phytochemicals such as glucosinolates, polyphenols, and carotenoids. Sprouts have been linked to a number of biological qualities, including anti-diabetic, anticarcinogenic, anti-inflammatory, and antioxidant effects. Broccoli sprouts contain sulforaphane, a compound that could result in cancer cell apoptosis, or programmed cell death, form new blood vessels that feed malignant cells, reduce inflammation, inhibit angiogenesis, reduce cancerous cell invasion and spread, lower susceptibility to cancer-causing substances, and increase certain detoxifying enzymes in the liver.^{6,7}

As a nutritious agricultural commodity, pea (*Pisum sativum L.*) is part of the Leguminosae family. Pea sprouts have important nutrients that can be subdivided into soluble and insoluble fibers, proteins (lysine and tryptophan), complex carbohydrates, folate, vitamin B, minerals such as potassium, iron, calcium, but lower contents of sodium, cholesterol, and saturated fat.⁸ Pea proteins range between 244 and 275 g kg^{-1} and starch contents range between 440 and 462 g kg^{-1} on a dry basis with soaking, germination, fermentation, pasteurization, and including advanced techniques such as pulse electric field or ultrasonication as different processing methods, having demonstrated an excellent efficacy by increasing the nutritional benefits and techno-functional attributes of peas and its proteins.^{9,10} Furthermore, pea sprouts include significant levels of antioxidant components like phenols, tocopherols, and carotenoids, which help the body to fight free radical damage that can result in disease. It has a number of health benefits, including lowering colon cancer prevalence, promoting type 2 diabetes, cardiovascular disease, and LDL-cholesterol. Pea sprouts naturally help diabetic individuals to monitor their blood sugar levels.¹¹ Therefore, the objective of this study is to ascertain the chemical constituents of broccoli and pea sprouts and their effects on biological, biochemical and immunity parameters of rats intoxicated with acrylamide.

Material and Methods

Materials

99% pure acrylamide (C₃H₅ON) was purchased from Sigma (Diesenhofen, Germany), and other analytical grade chemicals and kits utilized in the analyses were purchased from Merck, Darmstadt, Germany. 30 adult male albino Sprague Dawley rats (160± 5gm and 14-16 weeks old) were obtained from the medical research center of Jazan University, Jazan, Kingdom of Saudi Arabia. The basal diet comprised of 10% corn oil as a lipid source, 10% casein as a protein source, 1% vitamin mixture, 0.5% choline chloride, 5% cellulose as a fiber source, 69.5% corn starch, and 4% salt mixture. Broccoli (*Brassica oleracea L. var. italica*) and pea (*Pisum sativum L.*) were purchased from Food Research Department, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Giza, Egypt and were identified by Agricultural Research Station, Giza, Egypt.

Methods

Preparation of Broccoli and Pea Sprouts

Broccoli and pea sprouts were soaked at a volume of 1/3 to 1 cup of peas and broccoli seeds in cold water for 8-12 hours then drained of soak water, rinsed and drained with cold water every 8-12 hours. The sprouts were kept in a low light location and were harvested on day 11 when short roots had sprouted on the majority of plants.¹² The sprouts were heated

in the air oven dryer (Fill Pack Industries, Faridabad) at 40 °C for 6h., milled and sieved using a 0.6 mm sieve to obtain the sprouts powder.¹³

Determination of Chemical Constituents Total Flavonoid, Total Phenols and Antioxidants Activity of Broccoli and Pea Sprouts

Moisture, fat, protein and ash according to,¹⁴ dietary fiber was determined according to the methods of.¹⁵ Carbohydrate was ascertained by difference. Calorie values were calculated using values of 9 kcal/g for fat, 4 kcal/g for protein and carbohydrates as reported by.¹⁶ Mineral contents were ascertained using an atomic absorption spectrophotometer (PerKin Elmer Instrument Model 2380, 63110 rodgau, Germany), as detailed by.¹⁷ Vitamin C was determined using colorimetric methods as per the method of.¹⁸ Beta carotene and Vitamin E contents were determined in accordance with¹⁸ and,¹⁹ respectively. To determine total flavonoid, the aluminum chloride colorimetric method of²⁰ was used. The quantity of phenols in each of the tested sprouts was ascertained with Folin-Ciocalteu reagent using the methods by.²¹ The ABTS radical scavenging activity of triplicate tested sprout samples was conducted following the method of.²² DPPH radical scavenging activity was assayed in accordance with.²³ Estimating serum leptin concentration and serum insulin concentration were according to²⁴ and,²⁵ respectively.

Table 1: Composition of basal and sprout diets

Ingredients	Basal diet	5% broccoli sprouts	10% broccoli sprouts	5% pea sprouts	10% pea sprouts
Casein	10	10	10	10	10
Corn oil	10	10	10	10	10
Cellulose	5	5	5	5	5
Salt mixture	4	4	4	4	4
Vitamin mixture	1	1	1	1	1
Choline chloride	0.5	0.5	0.5	0.5	0.5
Broccoli sprouts	-----	5	10	-----	-----
Pea sprouts	-----	-----	-----	5	10
Starch	69.5	64.5	59.5	64.5	59.5

Induction Toxic with Acrylamide

During the experimental period, 25 rats received 2.5µg/kg body weight/ day to induce the acrylamide toxicity.²⁶

Experiment Biological Design

Preparation of the basic diet was conducted using the formula noted by.²⁷ This involved using vitamin mixture components as preferred by.²⁸ Formulation

of the salt mixture was conducted in accordance with.²⁹

Ethical approval was acquired by the Scientific Research Standing Committee of Jazan University, (REC-44/08/583). Every biological experiment was carried out at the medical research center of Jazan University. Individually housed rats (n = 30 rats) in wire cages in a room were kept under healthy conditions and maintained at 25±2°C. For acclimatization, before the experiment began, each rat was fed on basal diet for one week. The rats were then put into six groups (5 rats each), the first of which was given a basal diet as a negative control group while the second was given a basal diet and 2.5µg acrylamide /kg bw/d to induce acrylamide toxicity as a positive control group. The third and the fourth groups were treated with 2.5µg acrylamide /kg bw/d and fed on a basal diet with broccoli sprouts at the levels of 5 and 10% while, rats in groups 5 and 6 were treated with 2.5µg acrylamide /kg bw/d and fed on a basal diet with pea sprouts at the levels of 5 and 10% for 28 days as experimental period. The levels of different sprouts were added as replacement from the caloric content of added starch.

Biological Evaluation

Diet was recorded daily, and body weight was recorded weekly. The body weight gain (BWG), feed efficiency ratio (FER), and some organ weights were obtained based on³⁰ with the equations:

“Body weight gain = Final weight (g) – Initial weight (g)”

“Feed efficiency ratio (FER) = Body weight gain (g) / Feed intake (g)”

Blood Sampling and Organs

At the conclusion of each experiment, and after 12 hours fasting in every experimental group, blood samples were collected. This was done using the retro-orbital procedure using micro capillary glass heparinized tubes. Collection of blood samples were placed into clean, dry centrifuge tubes and left to clot for 30 minutes in a water bath (37 °C). To separate the serum, the blood was centrifuged at 3000rpm for ten minutes, and was aspirated into a clean cuvette tube and stored at – 20 °C for analysis.³¹ Analysis of all serum samples were performed to determine glucose according to³²

method. Alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) were measured according to³³ and³⁴ respectively. In addition, alkaline phosphatase (ALP) and total protein (Tp) were obtained based on³⁵ and ³⁶methods, respectively. Globulin (Glb) and albumin (Alb) were obtained by.³⁷ Uric acid, urea, and creatinine were ascertained in accordance with.^{38, 34} and ³⁹ Measurement of total cholesterol and triglycerides was performed in accordance with ⁴⁰and ⁴¹ respectively, HDL, VLDL-c and LDL-c were conducted using the method advocated by ⁴² and ⁴³ respectively. Glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), glutathione s-transferases (GSTs), total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured according to,^{29, 44, 45,46, 47, 48} and ⁴⁹ respectively. Monoaminoxidase (MAO), acetylcholinestrse (AChE), and creatine phosphokinase (CPK) were ascertained using the methods of ^{50, 51} and,⁵² respectively. ⁵³ was used to determine the immunoglobulin productions.

Simultaneously, the organs brain, liver, and pancreas were removed, washed in saline solution, wiped using filter paper, and weighed in accordance with.⁵⁴

Statistical Analyses

SPSS software (Statistical Package for the Social Sciences, version,²⁷ USA) was used to analyze the data, and was presented as mean ± SD, compared using a one-way ANOVA (analysis of variance) test followed by least significant difference (LSD) multiple range-test to determine significance of the intergroup. Significance was deemed to be a probability of p ≤0.05.

Results and Discussion

Chemical Constituents of Broccoli and Pea Sprouts

The proximate compositions of sprouts of broccoli and pea are shown in Table (2). The chemical composition reveals differences between the samples that are of statistical significance (p ≤0.05), according to the sprout types. The pea sprouts had significantly higher protein, fiber and ash than broccoli sprouts, while broccoli sprouts had high content in moisture, carbohydrates and energy content compared to pea content. In terms of fat content, there is no significant difference between the two sprouts. The increased metabolic activity

during the early germination phases, which stimulate protein synthesis, are attributed to this rise in protein levels. In dicotyledonous plants, reserve proteins are enzymatically hydrolyzed to produce amino acids and peptides, which are then used to create new hydrolytic enzymes.⁵⁵ The broccoli sprouts showed protein content from 16.16 to 34.4 g 100 g⁻¹ of dry matter,⁵⁶ and the obtained value is in the study range. In addition, broccoli sprouts displayed more protein contents than broccoli plant, with a value of

20.41 g 100 g⁻¹ dry matter.⁵⁷ Sprouts provide protein levels that are akin to high-protein diets. There was a reduction in total lipids seen during germination (Table 2). The aforementioned behavior is caused by a number of mechanisms in the lipid degradation metabolism by energy demand, which raises the respiratory rate.⁵⁵ The lipid content of broccoli sprouts has similarities to that reported by.⁵⁸ The ash content of broccoli and pea sprouts were found to differ significantly (p 0.05).

Table 2: Chemical constituents of 100g broccoli sprouts or pea sprouts

Nutrient components	Broccoli sprouts	Pea sprouts
Moisture	10.4 ^a ±1.23	8.43 ^b ±1.02
Protein	23.99 ^b ±0.22	34.93 ^a ±6.22
Fat	3.82 ^a ±0.03	4.04 ^a ±0.89
Dietary fiber	16.8 ^b ±1.65	18.23 ^a ±1.04
Ash	6.72 ^b ±0.98	8.54 ^a ±0.29
Carbohydrates	38.21 ^a ±2.86	25.83 ^b ±6.96
Total calories	283.18 ^a ±5.32	279.4 ^b ±3.41
Iron (mg)	11.89 ^a ±1.77	6.77 ^b ±0.65
Phosphorous (mg)	741.65 ^a ±9.64	206.55 ^b ±5.32
Potassium (mg)	898.06 ^a ±6.83	434.34 ^b ±11.22
Magnesium(mg)	537.89 ^a ±8.79	84.72 ^b ±7.23
Sodium (mg)	37.5 ^a ±1.65	24.63 ^b ±3.29
Calcium (mg)	228.07 ^a ±3.58	149.22 ^b ±4.32
Zinc (mg)	5.07 ^a ±0.67	1.34 ^b ±0.07
Selenium(µg)	9.12 ^a ±0.34	0.75 ^b ±0.02

Values are means ± SD.

The values are expressed means ± SD. Significant differences were found in the means from the same column with different litters (p≤0.05).

The ash content increases with germination time; the 11-day sprouts had the highest concentration (7.21 g 100 g of dry matter), a 44% increase over 3-day sprouts. These outcomes were almost identical to⁵⁶ in broccoli inflorescences with an ash content of 7.95 g 100 g¹. Most energy required to perform everyday tasks comes from consuming carbohydrates, which are primarily found in vegetables. Consuming fiber has been linked to a lower risk of diseases such as arteriosclerosis, hemorrhoids, colon cancer, and colitis.⁵⁹ The crude fiber content of broccoli sprouts fell considerably (p ≤0.05) with respect to their germination period. Sprouts had the lowest value (4.91 of dry matter). This is attributable to the stage of the germination's metabolic activation, which begins with the breakdown of reserve chemicals.⁵⁵

Regarding the lipid content, growing sprouts in open fields resulted in high lipid content (4.65%). Similar trends have been found for carbohydrate content, which may be because during germination the carbohydrate reserves, particularly in legume seeds, are consumed as a source of energy to begin the germination process, as well as various other processes, occurring in the seeds. In addition, in open fields, because of direct sunlight, the young seedlings can photosynthesize, leading to a content increase in carbohydrate.⁶⁰ However, ⁶¹ showed that peas had a low fat and carbohydrate; the decline is because they are used as an energy source during germination. The catabolism of lipids and carbohydrates to generate energy for seed germination may also contribute to the loss of the

dry weight and sprouted seeds give more energy as compared to normal seeds.⁶² reported that pea sprouts had high dietary fiber content and this increase was due to changes in the polysaccharides present in cell walls such as glucose, cellulose and mannose, suggesting that these changes were due to an increase in the plant's cell structure during germination and led to an increase in ash content.

From the same table, broccoli sprouts had significantly higher content from detected minerals compared to pea sprouts (on dry weight basis). Cruciferous vegetables are characterized by their mineral potential. Minerals play a vital part in a number of biological processes, which benefits both humans and plants. In the broccoli sprouts tested, the K and P concentrations were the highest, followed by that of Mg.⁶³ The increment in minerals content could be linked to the light exposure in the sprouting process and absorbing elements from media grown in open fields, as found by.⁶⁴ Yet, ⁶⁵ found that pea shoots had a higher content of phosphorous and potassium in comparison to other leafy green vegetables. The results confirmed that mineral contents were affected by environment and growing media.

Vitamins, Total Phenols, Total Flavonoid Contents and Antioxidants Activity of Broccoli and Pea Sprouts (Per100g)

Table (3) presents the broccoli and green pea sprout vitamins. Broccoli sprouts had significantly higher content of tested vitamins compared to pea sprouts. Vitamin E was the highest vitamin, followed vitamin A while pea sprouts had vitamin A, which was the highest compared to the other tested vitamins. During sprouting (germination), a number of enzyme systems became active and led to significant changes in the nutrition of pulses. Vitamin C, which is essentially absent in dry legume seeds, experienced an increase after sprouting.⁶⁵ However, ⁶¹ found that pea sprouts had a higher content of vitamin C, in contrast to other prepared leafy green veggies. The ascorbic acid in broccoli sprouts has been directly linked to the regulation of plant growth, especially in the early stages of embryo germination. As a result, sprout development, which includes vitamin C production reactivation, favors the vitamin C concentration in sprouts ⁶⁶. Sprouts of broccoli are a great source of fiber, sulforaphane, vitamin C, and other minerals. Flu causing viruses are fought

off by vitamin C, a potent natural antioxidant and immune system stimulator found in broccoli sprouts. It also has significant levels of another antioxidant, Vitamin A. It includes pro-vitamins such as carotene and zeaxanthin, which protect against macular degeneration of the retina and maintain skin integrity in the elderly.⁶³³

In the same, data show the total phenols, total flavonoid content, and antioxidants activity. The total phenols content was found to have a significant change ($p \leq 0.05$) between broccoli and pea sprouts. The pea sprouts showed high content of total phenols compared to broccoli sprouts, while the broccoli sprouts significantly increased in total flavonoid contents compared to pea sprouts. These results are similar to ⁶⁷ with an improved total phenols content in the older sprouts. The concentration differences of these plant metabolites fluctuate with variety, sample treatment, development stage, quantification method, and extraction process.⁶⁸ Broccoli sprouts total flavonoid content experienced a significant increase with the germination time, with the old sprouts increasing above 55% in flavonoid content.⁶⁹ The tissue age, variety, and environmental factors such as temperature and light could be the cause of variations in total flavonoid concentration between broccoli sprouts and the previously reported values. For example, the production of anthocyanins is frequently accelerated in aged tissues, and its quantity rises with light.⁷⁰ Pea seeds had a higher concentration of total phenols during germination than non-sprouted seeds, reaching day 6 maximum concentrations for bean sprouts of 70.04% higher than in the non-sprouted seeds. The pea's maximum TP concentration was attained on day 7, increasing by 55.85% from non-sprouted seeds, and phenolic compound synthesis was a reaction to the pea's germination⁷¹ found an increase in total flavonoid concentrations in the pea seed.⁶¹ The quantity of TP of the peas and their sprouts is more than that of fruits such as lychee, papaya, banana, pineapple and their by-products, demonstrating their capacity as a bioactive compound source.⁶¹ The DPPH procedure is a fast, simple, and appropriate method (in vitro) for evaluating the antioxidant capacities of vegetables and fruits.⁷² The quantification of total phenols and total flavonoids revealed similar behavior in the results of the DPPH radical scavenging capacity of pea and broccoli sprout (Table 3). When comparing the DPPH radical scavenging ability

of pea sprouts to broccoli sprouts, there was a progressive rise ($p > 0.05$). The greatest antioxidant levels were in sprouts, and multiple studies have shown a connection between total phenols content, vitamins, and antioxidant activity.^{73,74} Ferric reducing antioxidant power (FRAP) assay, ABTS radical

cation assay, or oxygen radical absorbance capacity (ORAC) assay can confirm the antioxidant properties of broccoli and pea sprouts, which is closely related to phenols compounds, and there were significant correlations between total phenols and ABTS.^{75,65}

Table 3: Vitamins, total phenols, total flavonoid contents and antioxidants activity of broccoli and pea sprouts (per100g)

Variables	Broccoli	Pea
Vit.C (mg)	51.62 ^a ± 8.54	32.65 ^b ± 1.34
Vit.E (IU)	749.88 ^a ± 10.43	6.65 ^b ± 0.82
Vit. A (IU)	210.45 ^a ± 9.76	199.87 ^b ± 5.72
Total phenols µgGAE/g	70.87 ^b ± 4.87	830.65 ^a ± 10.92
Total flavonoid µgGAE/g	110.64 ^a ± 6.83	67.34 ^b ± 0.99
DPPH (%)	39.76 ^b ± 7.93	79.65 ^a ± 5.32
ABTS (%)	67.65 ^a ± 1.03	49.65 ^b ± 2.04

Values are means ± SD.

The values are expressed means ± SD. Significant differences were found in the means from the same column with different litters ($p \leq 0.05$).

Table 4: Body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts

Groups	Parameters		
	BWG (g/28d) Mean ±SD	FI (g/day) Mean ±SD	FER (g/rat/day) Mean ±SD
Negative control (G1)	40.04 ^d ± 2.67	15.22 ^c ± 1.12	0.094 ^e ± 0.001
Positive control(G2)	63.32 ^a ± 0.91	20.22 ^a ± 0.71	0.112 ^a ± 0.002
5% broccoli sprouts (G3)	55.90 ^b ± 1.53	18.15 ^b ± 0.99	0.110 ^b ± 0.001
10% broccoli sprouts (G4)	47.40 ^c ± 0.64	16.42 ^c ± 0.76	0.103 ^d ± 0.003
5% pea sprouts (G5)	50.48 ^c ± 1.89	17.17 ^b ± 1.04	0.105 ^c ± 0.004
10% pea sprouts (G6)	41.68 ^d ± 2.05	15.67 ^c ± 0.78	0.095 ^e ± 0.001
3.77	1.63	0.001	

The values are expressed means ± SD. Significant differences were found in the means from the **same** column with different litters ($p \leq 0.05$).

The Table (4) results illustrate the effect of tested sprout levels on body weight gain (BWG), feed efficiency ratio (FER), and feed intake (FI) for rats treated by acrylamide as toxic material. It is apparent that the normal rats fed with a basal diet recorded normal BWG, FI/g/28 day and FER while the positive control group was significantly increased ($p \leq 0.05$) when compared to the other groups. The greatest

effects of the FI, BWG and FER in rats treated with tested toxic material was recorded for pea sprouts groups followed by broccoli sprouts groups and fed on diet containing 10% from tested sprouts seemed to reveal a high gradual decreasing of BWG, FI and FER followed by 5%. No significance was found between the sixth group and negative group. ACR is contained within a great deal of foods and

is produced when root vegetables or potatoes that contain the amino acid asparagine are cooked at a high temperature with foods that contain a high concentration of carbohydrates.⁷⁶

Acrylamide induces obesity and has been linked to abdominal obesity and weight gain in humans. However, there is an absence of clarity on the molecular mechanism whereby acrylamide can induce adipocyte differentiation and obesity through lipid metabolism.⁷⁷ Exposure to acrylamide may have an effect on thyroid function, and it is possible that the altered thyroid influences body weight. However, exposure to acrylamide has been linked to

hypothyroidism, which results in weight gain instead of weight loss.⁷⁸ Sprouts are high in fiber and low in calories, as well as an incredible source of protein. These factors make it an excellent weight-loss food. They help improve satiety and add to daily protein requirements. Broccoli sprouts are rich in a range of nutrients, including fiber, vitamin A, vitamin C, and sulforaphane. Sulforaphane results in 20% fat loss by altering gut bacteria and increasing mitochondria in fat in mice.⁶⁹ Pea sprouts are an excellent source of micronutrients, they provide protein which promotes satiety, low fat and fiber as well as providing vitamins C, A, K, and other vital vitamins and minerals.⁷¹

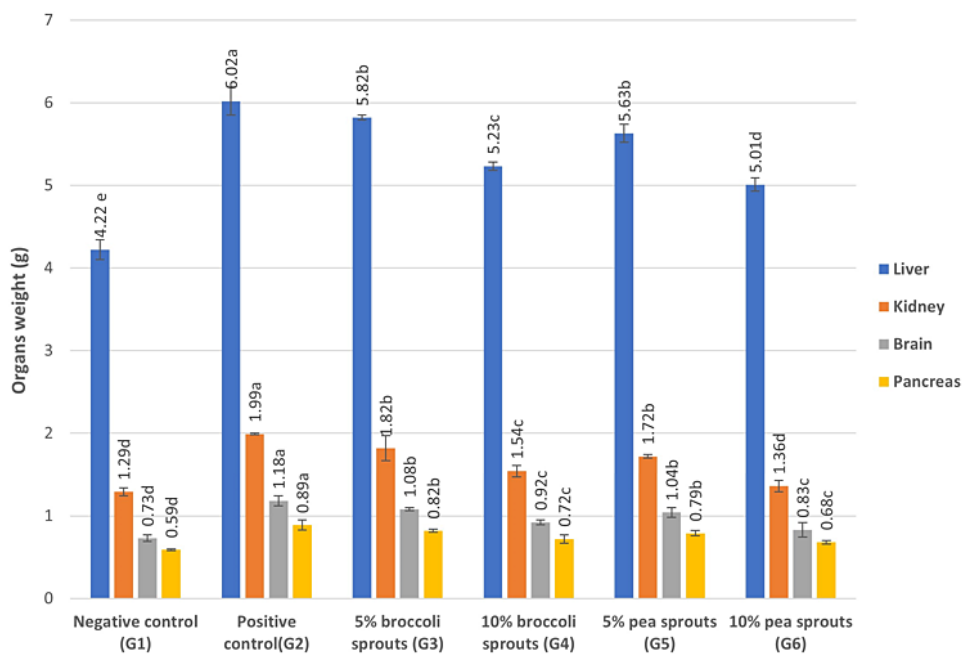


Fig. 1: Some organs' weights of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts (g). The values are expressed means \pm SD. Significant differences were found in the means from the same color with different letters ($p \leq 0.05$).

The results in figure (1) show the effects of levels of pea and broccoli sprouts in rats treated with acrylamide on liver, kidney, brain and pancreas weights. The results showed that the weight of organs significantly increased ($p \leq 0.05$) in the positive control group in comparison to other groups. The best level was 10% which had a high effect on organs weight, but they remained significantly higher ($p \leq 0.05$) than the negative control group, except kidney weight. The obtained results were matched

with the results of ⁷⁷ who found that acrylamide at the dose 2 μg kg/ body weight / day per 35 days caused a significant increase in relative brain and liver weight. Sprouts are rich in many important nutrients which, depending on the type of sprout, contain high levels of carbohydrates, protein, dietary fibers, folate, magnesium, phosphorus, and vitamin K. They also possess antioxidant and antimutagenic activities which protect the internal organs from free radical and toxic material.⁶⁹ Germination is a simple

and inexpensive way to improve nutritive value. A diet that is high in broccoli sprouts offers an excellent source of vitamin C, indole-3-carbinol, flavonoids, sulforaphane, and minerals and might lower the risk of organ diseases, especially cancer.⁵⁷ Peas contain bioactive compounds including micro-nutrients, macronutrients, and phytochemicals. Phytochemicals, usually polyphenols, such as flavonoids and phenolics compounds, are known to provide anti-oxidative and anti-inflammatory properties in internal organs.⁷¹

The results in table (5) show the effects of tested sprouts levels on serum glucose, insulin and leptin hormones. As revealed in the table, group 6 had the best glucose level and tested hormones; this group was toxic with acrylamide and fed on 10% pea sprouts followed by the same level of broccoli sprouts. There were significant ($p \leq 0.05$) changes between both controls and different tested groups.⁷⁹ discovered that there is a large positive dose-related link between acrylamide exposure and an increase in fasting plasma glucose⁷⁷ indicated that insulin resistance was related to acrylamide exposure and a decrease in insulin level. Male rats given acrylamide at doses of 25 mg/kg b.w./d and 50 mg/kg b.w./d

reduced their beta cells by 15% and 25%, impairing normal blood levels of glucose and insulin.⁸⁰ The concentration of plasma leptin increases proportional to body fat mass and regulates food intakes and energy outlay by using brown fat for energy and excessive hunger to maintain body fat stores. Leptin is known to inhibit insulin secretion by PI3K-dependent activating phosphodiesterase 3B (PDE3B) and by decreasing the level of cAMP, therefore inhibiting the protein kinase A (PKA) pathway and is a regulator of Ca^{2+} channels and exocytosis.^{81, 82} Sprouts can help to control blood sugar levels because they contain lower levels of carbohydrates, which can help to regulate insulin levels. Pea sprouts can lower blood glucose levels. The fiber and protein content of peas slows digestion and smoothens the body's blood sugar levels.⁸³ Broccoli has a very low Glycemic Index and can also help regulate blood sugar levels. Furthermore, broccoli contains a lot of fiber, which can help regulate appetite and maintain stable blood sugar levels. Research has shown that sulforaphane-rich broccoli extract has potent anti-diabetic benefits, lowering blood sugar and oxidative stress indicators, and improving insulin sensitivity.⁸⁴

Table 5: Serum glucose, insulin and leptin hormones of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts

Groups	Parameters		
	Glucose mg/dl Mean \pm SD	Insulin μ U/mL Mean \pm SD	Leptin ng/mL Mean \pm SD
Negative control (G1)	104.66 ^f \pm 5.94	17.43 ^a \pm 1.11	12.34 ^f \pm 1.02
Positive control(G2)	206.54 ^a \pm 7.04	6.18 ^f \pm 0.68	25.08 ^a \pm 0.99
5% broccoli sprouts (G3)	185.09 ^b \pm 4.51	7.80 ^e \pm 0.94	22.18 ^b \pm 1.04
10% broccoli sprouts (G4)	157.12 ^d \pm 6.21	10.87 ^c \pm 1.21	18.50 ^d \pm 0.87
5% pea sprouts (G5)	175.33 ^c \pm 5.62	9.05 ^d \pm 0.93	20.40 ^c \pm 1.28
10% pea sprouts (G6)	135.29 ^e \pm 4.81	12.07 ^b \pm 1.04	16.51 ^e \pm 0.08
LSD	7.65	1.24	1.77

The values are expressed means \pm SD. Significant differences were found in the means from the **same** column with different litters ($p \leq 0.05$).

Figures (2 ,3) shows that significant increases occurred in the concentration of the liver enzymes (AST, ALT and ALP) and total protein which equal the sum of albumin and globulin in the blood serum in the

positive control group in comparison to the negative control group. The groups which were toxic with AA and treated with tested sprouts showed significant improvement in tested parameters in comparison to

positive control group but were still higher than the negative control group. The 10% level from tested sprouts had the greatest effect followed by 5%, and the pea sprouts recorded a higher effect than the broccoli sprouts. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are enzymes, which when altered signify liver damage. Acrylamide causes liver damage and increases the levels of those enzymes.⁸⁵ Sprouts contain phenols and vitamins which play a vital role as a strong antioxidant in

scavenging free radicals, reducing cell toxicity and death, and are also anticarcinogenic. Solforavan compound extracted from broccoli decreases liver damage by causing a decrease in the amounts of AST, ALT, and ALP levels and to activate Nrf2/Keap pathway.⁸⁶ Peas have low-fat-rich protein, high fiber and high content of antioxidant components mainly including phenols, tocopherols, and carotenoids, which significantly alleviate oxidative stress in the liver.⁸⁷

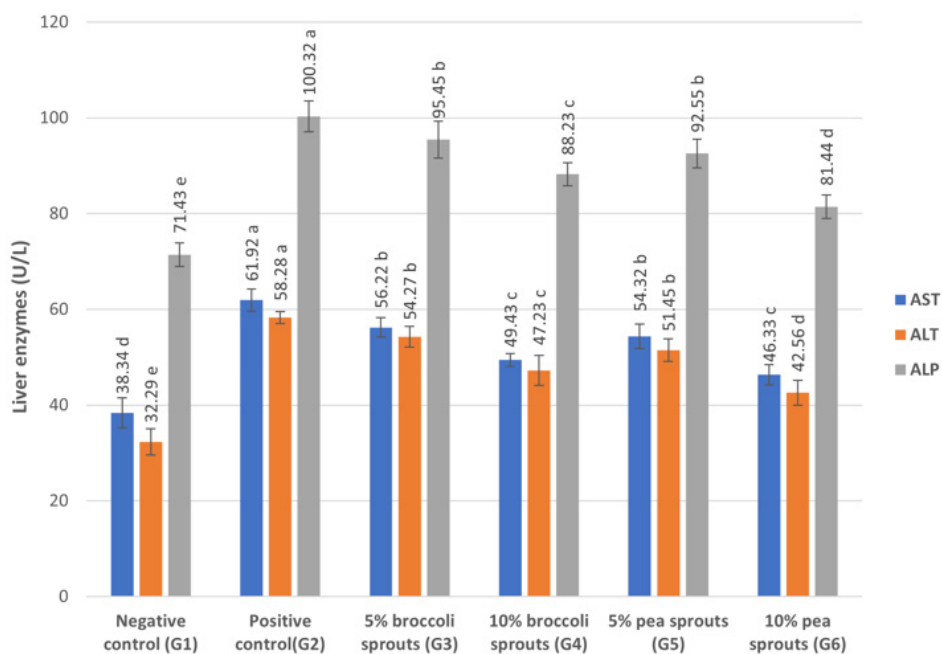


Fig. 2: ALT, AST, ALP of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts. The values are expressed means \pm SD. Significant differences were found in the means from the same color with different letters ($p \leq 0.05$).

The results in table (6) show that the positive control group's levels of uric acid, urea, and creatinine were significantly higher ($p > 0.05$) than those of the negative control group. When compared to the toxic positive control group, all toxic rats with AA who were fed a diet that contained 5% and 10% broccoli and pea sprouts showed significantly lower kidney function levels. The group 6 toxic rats fed 10% pea sprouts received the best care. The 10% of examined sprouts had no significant difference between the groups. The findings showed that oral exposure to acrylamide through drinking water or gavages causes chronic liver disease, hepatic

insufficiency, and kidney damage, leading to primary immunodeficiency and immune system activation after the exposure.⁸⁸ The kidneys of the acrylamide-treated groups exhibited glomerular tuft degradation and lymphocyte infiltration. The epithelial lining of the renal tubules had degenerative alterations, which were followed by cell rupture, necrosis, and interstitial blood vessel congestion. The renal tubules also lost their brush boundaries and became vacuolated.⁸⁹ Compounds found in broccoli could help to treat or halt the course of CKD. Numerous phytochemicals can increase NRF2 and offer oxidative protection. Glucosinolates(-thioglucoside

N-hydroxysulfates), such as the glucoraphanin found in broccol.⁹⁰ Treatment with pea sprouts can lower blood pressure and provide protection against hypertensive kidney injury by boosting anti-inflammatory and antioxidant defense responses and regulating the renin-angiotensin system.⁸⁷

Uric acid, urea, and creatinine levels in the serum of the administered rat were considerably lower ($P < 0.05$) than in the control group. Reductions in the concentrations of uric acid, urea, and creatinine were discovered to be dose-dependent.

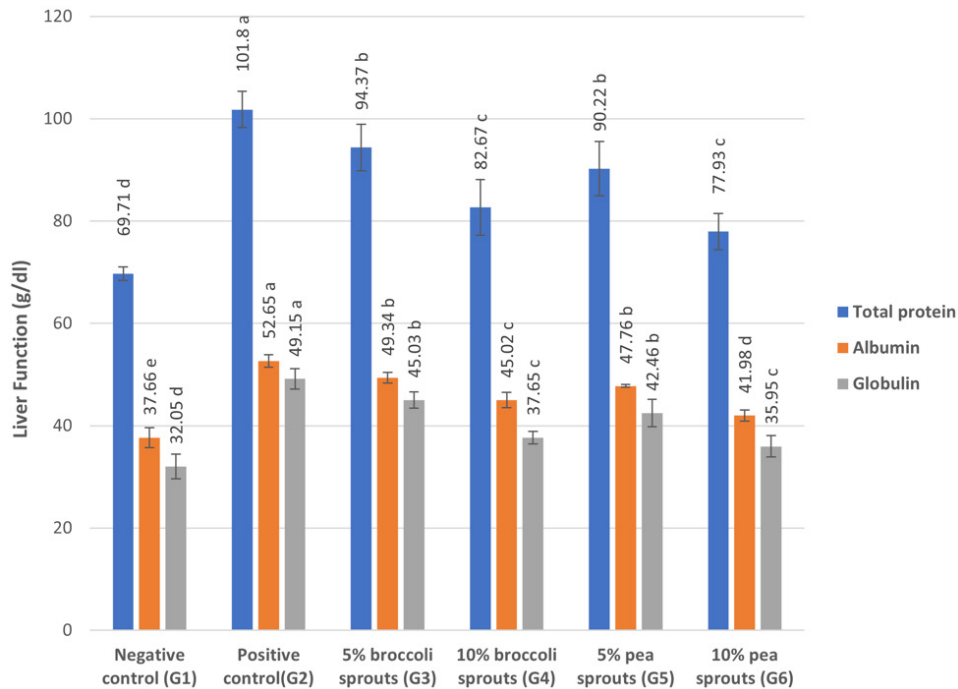


Fig. 3: Total protein, albumin and globulin of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts. The values are expressed means ± SD. Significant differences were found in the means from the same color with different litters ($p \leq 0.05$)

Table 6: Affecting the levels of broccoli and pea sprouts on creatinine, urea, and uric acid of rats treated with acrylamide

Groups	Parameters		
	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Negative control (G1)	0.73e±0.06	18.34d±2.05	1.23d±0.18
Positive control(G2)	1.16a±0.04	29.05a±2.31	2.91a±0.29
5% broccoli sprouts (G3)	1.06b±0.01	25.80b±2.12	2.45b±0.01
10% broccoli sprouts (G4)	0.98c±0.002	20.22d±2.12	1.96c±0.18
5% pea sprouts (G5)	0.97c±0.08	24.75c±1.23	2.21b±0.21
10% pea sprouts (G6)	0.88d±0.02	19.05d±2.43	1.71c±0.061
LSD	0.090	2.89	0.28

The values are expressed means ± SD. Significant differences were found in the means from the **same** column with different litters ($p \leq 0.05$).

Table (7) illustrates the mean values of serum lipid profile concentration in both of controls and toxic groups with AA and treated with levels of tested sprouts. There were significant ($p \leq 0.05$) increases of TC, TG, (LDL-c), (VLDL-c) and aside from a significant ($p \leq 0.05$) reduction of (HDL-c) of toxic control group with AA in comparison to negative control group rats. Moreover, every toxic rat fed on diet that contained 5% and 10% tested sprouts revealed marked decreases of TC, TG, (LDL-c) and (VLDL-c) as well dramatic increases of (HDL-c). The best results were for group 6 (rats fed on 10% pea sprouts) where it had a potent effect. No significant differences were found between groups fed on 10% broccoli sprouts and 5% pea sprouts for TC, TG, (LDL-c) and (VLDL-c). The data obtained by 91 shows how AA affected the lipid profile in the blood, significantly raising TC and TG levels while significantly reducing HDL-C levels. The involvement of AA in lipid peroxidation can therefore account for the findings. High plasma oxidative stress indicators were negatively connected with low HDL and favorably correlated with elevated plasma triglycerides.

By inhibiting lipoprotein lipase and the reduction in VLDL clearance in adipocytes caused by impaired

insulin signaling, AA exposure could result in atherogenic dyslipidemia. Additionally, the amount of free fatty acids (FFA) in the liver is increased, which acts as a substrate for the production of TG and causes hypertriglyceridemia. The formation of Apo B, the primary apoprotein of VLDL particles, is also stabilized by the FFA.^{92, 93} Numerous trials have found that consuming sprouts decreases cholesterol in those with diabetes or obesity. One study found a decrease in triglycerides and "bad" LDL cholesterol and an increase in "good" HDL cholesterol.⁹⁴ shows that broccoli sprouts could make improvements to lipid profiles and blood glucose. Plasma LDL-C was also significantly down regulated with intake of high glucoraphanin broccoli. According to,⁹⁵ consumption of broccoli sprouts can reduce cardiovascular diseases and enhance lipid-related metabolic markers. 96 discovered that, compared to nonfermented pea, fermented pea significantly reduced the serum levels of TC, TG, and LDL in mice. This could be because aminobutyric acid found in fermented peas helps hypercholesterolemic mice by lowering their lipid levels. Additionally, it contains lectins, oligosaccharides, folate, selenium, zinc, saponins, and selenium, all of which reduce the oxidative stress that is strongly linked to hyperlipidemia.⁹⁷

Table 7: Lipid profile of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts

Group	Parameters				
	Triglycerides (mg/dl)	Total cholest-erol (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Negative control (G1)	97.61 ^e ± 4.05	99.68 ^e ± 3.12	50.74 ^a ± 2.55	29.42 ^e ± 2.58	19.52 ^d ± 0.45
Positive control (G2)	129.4 ^a ± 5.03	137.14 ^a ± 3.11	39.62 ^c ± 2.34	71.64 ^a ± 1.82	25.88 ^a ± 0.55
5% broccoli sprouts (G3)	125.75 ^b ± 3.65	134.3 ^b ± 2.33	44.31 ^b ± 2.35	64.84 ^b ± 2.93	25.15 ^a ± 0.25
10% broccoli sprouts (G4)	118.53 ^c ± 3.02	126.5 ^c ± 1.78	48.46 ^a ± 2.64	54.33 ^c ± 1.51	23.71 ^b ± 0.32
5% pea sprouts (G5)	121.4 ^c ± 3.85	127.58 ^c ± 2.89	45.62 ^b ± 2.55	57.68 ^c ± 3.88	24.28 ^b ± 0.13
10% pea sprouts (G6)	111.32 ^d ± 2.65	122.53 ^d ± 2.19	49.63 ^a ± 1.89	50.64 ^d ± 2.78	22.26 ^c ± 0.13
LSD	6.75	3.32	2.67	3.91	1.45

The values are expressed means ± SD. There is a significant difference in the means from the same column with different litters ($p \leq 0.05$).

Table (8) shows that the positive control group's activating levels of GPX, SOD, and CAT were considerably lower ($p \leq 0.05$) than those of the

negative control group. All rats given acrylamide treatment and diets containing 5 and 10% broccoli and pea sprouts had steady increases in antioxidant

levels that were considerably ($p > 0.05$) higher than those of the positive control group. Group 6 received the best therapy, which raised the antioxidant enzymes. The table also shows that, when positive controls were compared to negative controls, there were significant declines in the levels of GST and TAC as well as significant increases in MDA, but all toxic rats fed diets containing the tested sprouts experienced a significant improvement in GST levels.⁹⁸ Tested sprouts have a high antioxidant capacity and are rich in polyphenols, which impact upon antioxidant enzyme activities in vivo, for example an increase in SOD and GSH activities and a decrease in MDA levels.⁹⁹ Broccoli sprouts have high antioxidant effects in human cells as a

result of several protective compounds. They contain 10-100x more enzymes than fruits and vegetables when eaten without germination. They are rich in glucoraphanin, which is changed to another compound called sulforaphane during digestion. This chemical has antioxidant effects which might help prevent cancer and liver problems.¹⁰⁰ Pea sprouts contain high levels of flavonoids and phenolic compounds, which are essential in, hepatic impairments, reducing inflammation in hepatic cells apoptosis, and structural injury in the liver while also inhibiting both types of structural injury via degradation of oxidative stress, DNA damage to the liver, and malondialdehyde (MDA), which is linked to liver damage caused by acrylamide.^{101, 102.}

Table 8: Glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferases (GST), total antioxidant capacity (TAC) and malondialdehyde (MDA) of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts

Group	Parameters					
	GPX (ng/dl) (U/L)	SOD (mmol/L)	CAT (mmol/L)	GST (nmol/L)	TAC (nmol/L)	MDA
Negative control (G1)	83.73 ^a ±3.21	50.65 ^a ±2.9	73.04 ^a ±5.67	31.97 ^a ±2.49	1.63 ^a ±0.09	13.97 ^a ±1.88
Positive control(G2)	58.43 ^d ±3.10	32.05 ^d ±4.19	42.83 ^d ±3.41	20.20 ^d ±4.08	0.83 ^e ±0.05	35.21 ^a ±4.05
5% broccoli sprouts (G3)	63.54 ^c ±3.66	39.28 ^c ±3.52	48.09 ^d ±4.22	23.11 ^c ±2.06	0.99 ^d ±0.14	30.78 ^b ±2.74
10% broccoli sprouts (G4)	70.89 ^b ±3.14	44.77 ^b ±3.21	56.38 ^c ±6.17	28.16 ^b ±1.73	1.33 ^b ±0.25	25.58 ^c ±4.22
5% pea sprouts (G5)	65.48 ^c ±3.78	41.74 ^b ±3.82	52.15 ^c ±6.54	25.84 ^b ±1.22	1.30 ^c ±0.02	27.57 ^b ±3.44
10% pea sprouts (G6)	73.16 ^b ±2.86	47.88 ^a ±2.43	61.55 ^b ±6.53	30.54 ^a ±2.67	1.38 ^b ±0.002	20.67 ^d ±2.45
LSD	4.21	3.86	4.76	2.89	0.07	3.85

The values are expressed means \pm SD. There was a significant difference in the means from the same column with different litters ($p \leq 0.05$).

Table (9) shows alterations to the serum parameters, including brain enzymes. A significant decrease was found in the activities of MAO and AChE in the positive control group, while CPK activities had a significant increase in the same group compared to the negative control group and the groups fed on 5% and 10% tested sprouts. The pea sprouts had a significant effect compared to broccoli sprouts. The levels of 10% of tested sprouts recorded a high effect compared to 5%. There was no significant variation between the 10% pea sprouts group and negative control group. Acrylamide interacts with various biochemical processes such as acetylcholine metabolism and therefore can act as a potential

etiopathogenic cofactor. It causes AChE inhibition and decreased MAO activity, which may represent the mode of action by which AA contributes to pathological processes inducing neurotoxicity.⁹⁸ Sprouts have high levels of omega-3 fatty acids and support neuronal health and decrease the risks of heart attack and stroke. Pea and broccoli sprouts have high levels of magnesium and calcium, which support bone and muscle health. Magnesium is crucial for vessel and brain health. Pea sprouts are an excellent source of dietary fiber, vitamin A, C and E, and potassium. Glycosylated quercetin derivatives are the primary flavonoid compounds of pea sprouts and protect the brain from damage

and keep its enzymes within normal range. Broccoli sprouts are packed with vitamin K which can help limit the extent of brain neuron damage

and help delay or prevent the onset of Alzheimer's disease.^{100, 96}

Table 9: The brain enzymes of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts

Group	Parameters		
	MAO U/ml	AChE U/ml	CPK U/l
Negative control (G1)	21.03 ^a ± 1.56	6.48 ^a ± 1.08	22.57 ^d ± 2.84
Positive control(G2)	10.64 ^d ± 1.93	1.22 ^e ± 0.84	34.06 ^a ± 2.89
5% broccoli sprouts (G3)	13.67 ^c ± 1.17	2.79 ^d ± 0.06	30.19 ^b ± 2.54
10% broccoli sprouts (G4)	17.98 ^b ± 2.66	4.59 ^c ± 0.98	26.34 ^c ± 2.81
5% pea sprouts (G5)	16.59 ^b ± 1.05	3.01 ^d ± 0.76	27.88 ^b ± 2.61
10% pea sprouts (G6)	20.99 ^a ± 4.76	5.31 ^b ± 0.79	23.07 ^d ± 2.61
LSD	2.92	0.99	2.88

The values are expressed means ± SD. There was a significant difference in the means from the same column with different litters (p≤0.05).

Table 10: IGA, IGG and IGM of rats treated with acrylamide by affecting the levels of broccoli and pea sproutss

Group	Parameters		
	IGA (mg/dl)	IGG (mg/dl)	IGM (mg/dl)
Negative control (G1)	140.44±9.72 ^a	610.3±7.92 ^a	150.63±9.08 ^a
Positive control(G2)	80.73±5.82 ^e	445.74±9.06 ^f	70.37±7.69 ^f
5% broccoli sprouts (G3)	91.95±4.89 ^d	467.93±8.34 ^e	79.75±5.91 ^e
10% broccoli sprouts (G4)	102.05±7.44 ^c	507.65±10.04 ^c	95.91±5.67 ^c
5% pea sprouts (G5)	97.34±10.22 ^c	487.87±10.94 ^d	86.97±6.48 ^d
10% pea sprouts (G6)	116.98±9.45 ^b	527.67±8.99 ^b	107.67±5.48 ^b
LSD	6.89	10.87	5.32

The values are expressed means ± SD. There are significant differences in the means from the same column with different litters (p≤0.05).

IGA, IGG and IGM of rats treated with acrylamide by affecting the levels of broccoli and pea sprouts Table (10) shows higher values in IGA, IGG and IGM for the negative control in comparison to the positive one. Significant differences were found in the mean values of tested parameters across the treatment groups (p ≤ 0.05). Highest values of tested parameters were found in the 10% pea sprouts group. Sprouts contain phenol compounds which are vital in defense responses, including

anti-inflammatory, anti-aging, antioxidant and anti-proliferative activities. Broccoli and pea sprouts have higher vitamin C content, making them potent stimulants for the body's white blood cells in order to fight disease and infection, thus, helping to build immunity. Also, they have a high number of phenols which act as an antioxidant by reacting with free radicals by transfer of a single electron, hydrogen atom transfer, chelation of transition metals, and sequential proton loss electron transfer. They

also contain flavonoids which inhibit a number of enzymes such as phosphodiesterase, xanthine oxidase, aldose reductase and regulate different hormones such as thyroid hormones, estrogens, and androgens.^{103, 104}

Conclusions

Consuming broccoli sprouts and pea sprouts is an economical, intelligent, and simple option to eat healthily. The broccoli and pea sprouts analyzed in this research are low in fat and rich in protein. The sprouts also have high contents of flavonoids, polyphenols, a high DPPH radical scavenging capacity and ABTS. The results indicate the benefit of tested sprouts consumption for anti-inflammatory activity, improved brain enzymes, decreased biological parameters (body weight gain, feed intake

and some organs weight) and decreased the levels of tested biochemical parameters (fasting blood glucose, liver enzymes, kidney functions, lipid profile and MDA).

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Conflict of Interest

The authors declare that there is no actual or potential conflict of interest.

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